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AUTHOR(S):

WATANABE, Takashi; KOSHIJIMA, Tetsuo

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Application of APT Pulse Sequence to Facilitate ^{13}C -NMR Analysis of Lignin^{*1}

Takashi WATANABE^{*2} and Tetsuo KOSHIJIMA^{*2}

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Abstract—A convenient J-modulation pulse sequence, Attached Proton Test (APT) technique has been applied to the acetylated milled wood lignin from akamatsu wood. Signals originating from *p*-acetylated guaiacyl nuclei were estimated by comparing two types of acetylated milled wood lignin, *p*-methylated (MWLma) and *p*-acetylated (MWLa) preparations from the softwood. Reaction of diazomethane with cinnamaldehydes in the lignin was confirmed from the APT spectra of both acetylated fractions.

Keywords: nmr, APT, lignin, akamatsu

1. Introduction

In the last few years, a number of nuclear magnetic resonance (NMR) techniques have been developed to analyze fine structures of synthetic and natural macromolecules. In particular, two-dimensional pulse sequences have been widely used for assigning the complex molecules. However, these excellent new pulse sequences have not been applied to DHP and lignins except for INADEQUATE¹⁻³⁾, C-H COSY⁴⁾ and C-H 2DJ⁵⁾ pulse sequences, and most of liquid-state ^{13}C -NMR spectra of lignin have been measured by conventional complete decoupling (COM), gated decoupling without NOE (NNE)⁶⁻⁸⁾, and DEPT techniques^{9,10)}. Thus we can find regrettably only a few applications of the sophisticated pulse sequences in the field of wood chemistry. Consequently, there is much confusion of the signal assignments in this field, because solvent and steric compression^{11,12)} effects complicate the signal assignments.

In this study, we have focused on a simple one-dimensional technique to discriminate carbon multiplicity in lignins in view of applying a spin-echo technique to the routine NMR analyses of lignins. We used Attached Proton Test (APT) technique¹³⁾ for this purpose because this technique makes possible to discriminate carbon multiplicity with much greater ease than the polarization transfer pulse se-

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^{*2} Research Section of Wood Chemistry.

quences such as INEPT, DEPT, POMMIE and PREP methods. The APT technique is not sufficient for the decisive signal assignments of lignin, but is effective for enhancing signal resolution. Applicability of the APT technique to milled wood lignin (MWL) acetates from akamatsu wood is reported.

2. Experimental

2.1 Preparation of acetylated milled wood lignin (MWLa)

Milled wood lignin from akamatsu (*Pinus densiflora* Sieb. et ZUCC.) was prepared according to the procedure of Björkman¹⁴⁾. Acetylation of the MWL was carried out with pyridine/acetic anhydride (1:1, v/v) at 40°C for 18 hr. After acetylating, the lignin was recovered by precipitation from diethyl ether, purified by successive washings with the same solvent, and dried *in vacuo*.

2.2 Preparation of *p*-methylated lignin acetate (MWLma)

Milled wood lignin was methylated with diazomethane at room temperature for 48 hr in a diethyl ether-methyl alcohol mixture (2:1, v/v). After evaporating, the methylated sample was acetylated as in the case of MWLa.

2.3 NMR spectra

Proton decoupled ^{13}C -NMR spectra were obtained from 15 to 20% solutions

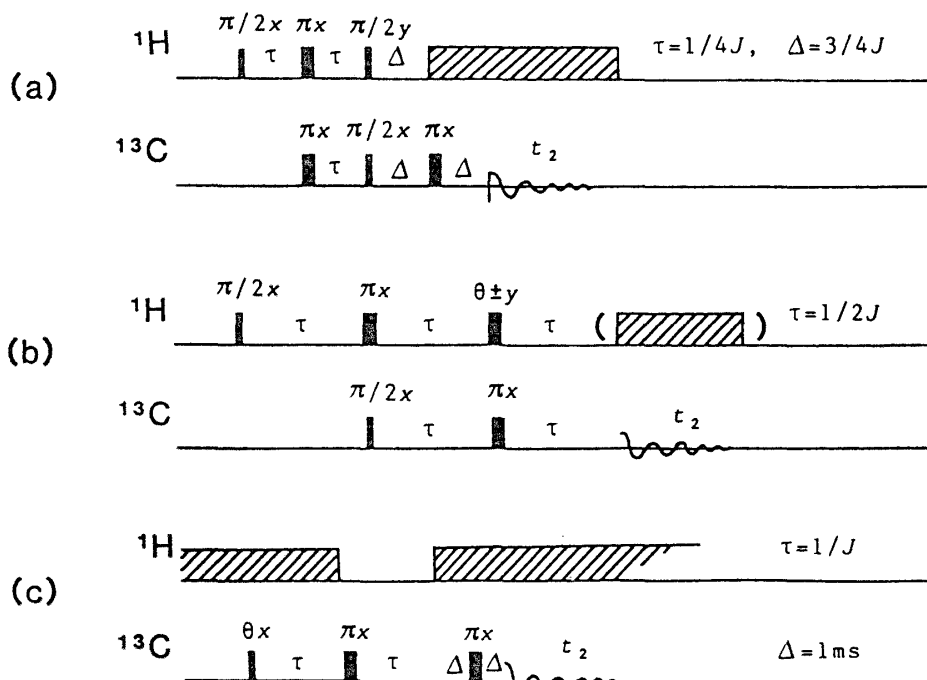


Fig. 1. Pulse sequences for the proton polarization transfer and Attached Proton Test (APT) methods. (a): INEPT/REFOCUS, (b): DEPT, (c): APT

Table I. Chemical shifts and main assignments for akamatsu acetylated lignin fraction (MWLa)

Signal No.*	ppm	Main assignments**	Intensity	Atomic groups	Ref.
1	194.2	α -CO and γ -CHO in cinnamaldehyde	m	C, CH	15
2	191.1	α -CHO	w	CH	15
3	170.9	Primary acetoxy-CO	vs	C	15
4	170.1	Secondary acetoxy-CO	vs	C	15
5	169.1	Phenolic acetoxy-CO	s	C	15
6	153.3	Ga-3 (α -OR); C α in cinnamaldehyde	m	C, CH	15, 17
7	151.3	Ga-3 (α -OAc)	s	C	15
8	150.0	Ge-3 (α -OR)	m	C	15
9	148.8	Ge-3 (α -OAc); Ge-4 in β -O-4 (threo)	m	C	15, 18
10	148.2	Ge-4 in β -O-4 (erythro)	m	C	18
11	144.9	C-4' in phenylcoumaran	m	C	15
12	140.5	Ga-4, Ga-1 (α -OR)	m	C	15
13	137.1	Ga-1 (α -OAc)	w	C	15
14	136.3	Ga-1 (α -OAc) in β -arylethers	m	C	18
15	132.8	Ge-1 (α -OR)	m	C	15
16	132.0	Ge-1 (α -OAc); C β in cinnamaldehyde	m	C, CH	15
17	130.0	C-1 in phenylcoumaran; H2/6	m	C, CH	15, 17
18	128.8				
19	123.4	Ga-5	s	CH	15
20	120.5	Ge-6	s	CH	15
21	118.9	Ge-5 (α -OAc); Ga-6	s	CH	15
22	117.6	C-6' in phenylcoumaran; Ge-5 (α -OR)	w	CH	15, 17
23	113.9	Ge-2 ($\alpha=0$)	w	CH	17
24	112.4	Ge-2; Ga-2	s	CH	15
25	111.2				
26	88.0	C α in phenylcoumaran	m	CH	15
27	85.8	C α in pinoresinol	m	CH	15
28	83.2	C α in β -ethers	w	CH	18
29	80.5	C β in β -O-4 (threo)	m	CH	5
30	80.0	C β in β -O-4 (erythro)	m	CH	5
31	75.1	C α in β -O-4 (threo)	m	CH	5
32	74.5	C α in β -O-4 (erythro)	m	CH	5
33	72.9	unknown	w	CH	—
34	72.2	C γ in pinoresinol	w	CH ₂	15
35	65.9	C γ in phenylcoumaran and cinnamyl alcohol	s	CH ₂	17, 18
36	63.9	C γ in β -1 and α , β -bis-O-4	s	CH ₂	15
37	63.5	C γ in β -O-4 (threo)	s	CH ₂	5
38	63.0	C γ in β -O-4 (erythro)	m	CH ₂	5
39	56.2	OMe	vs	CH ₃	15
40	51.0	C β in β -1 and phenylcoumaran	m	CH	15
41	20.7	CH ₃ in acetyl	vs	CH ₃	15

*; See Fig. 2, **; Abbreviations: guaiacyl (G), *p*-hydroxyphenyl (H), acetylated (a), etherified (e), very strong (vs), strong (s), medium (m), weak (w).

in acetone- d_6 on a Varian XL-200 Spectrometer operating at 50.3 MHz (10 mm tubes; TMS as internal standard). Each spectrum was recorded with 0.666 sec. acquisition time at room temperature. The complete decoupling spectra were obtained from 12 hr scans with 40° flip angle and 1 sec. pulse delay time. The APT spectra were obtained from 16 hr scans with 75° and 180° pulses, 6 m sec. τ delay time, 1 m sec. short delay (4) time and 0.8 sec. repetition time.

3. Results and Discussion

The Attached Proton Test (APT) technique is a method for discriminating between CH_3 , CH_2 , CH and quaternary groups by modulation with a second spin echo¹³⁾. This method is well suited for the routine analyses on account of its sim-

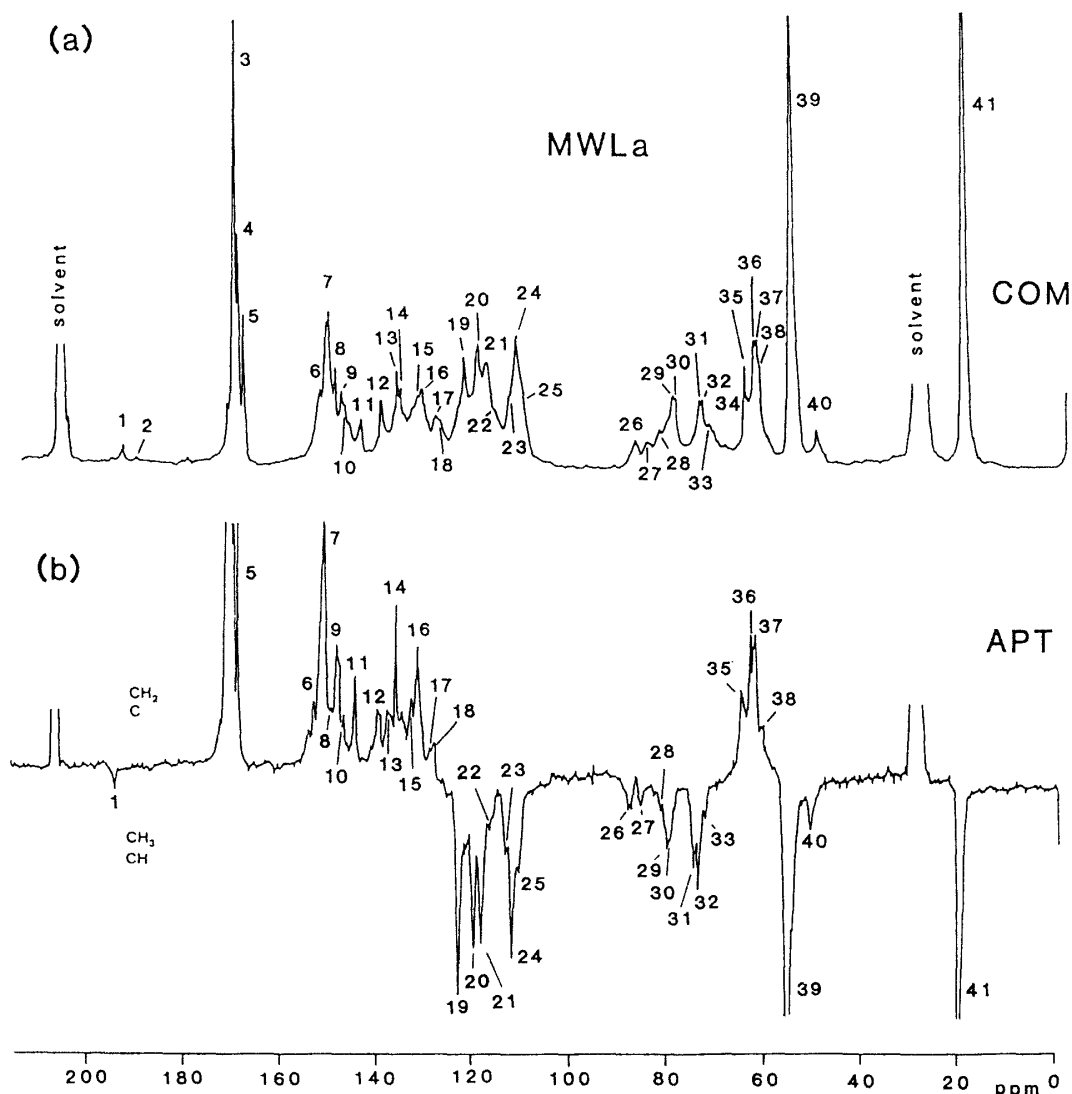


Fig. 2. ^{13}C -NMR spectra of acetylated MWL fraction (MWLa). (a): Conventional complete decoupling (COM) method, (b): Attached Proton Test (APT) method.

plicity of pulse sequences, as shown in Fig. 1.

The ^{13}C -NMR spectra of the acetylated MWL (MWLa) from akamatsu wood, measured by the conventional complete decoupling (COM) and Attached Proton Test (APT) techniques, are shown in Fig. 2. Each signal in the spectra was assigned (Table I) by referring recent articles^{15,16)}, in which NMR spectra were measured for acetone- d_6 solution. Supplementary informations were obtained from the original papers using an acetone- $\text{d}_6/\text{D}_2\text{O}$ mixture (9:1)^{5,17)} and chloroform- d ¹⁸⁾. As shown in Fig. 2, each signal in the APT spectrum was separated into upper and lower phases due to the multiplicity of the carbon atoms. In addition, it was found that signal resolution of the APT spectrum was enough to analyze structural features of the molecules. For instance, signal No. 21, which almost overlapped with No. 20 in the COM spectrum, was shown to be separated each other in the APT spectrum.

To discriminate carbon atoms in *p*-etherified units from those in *p*-acetylated nu-

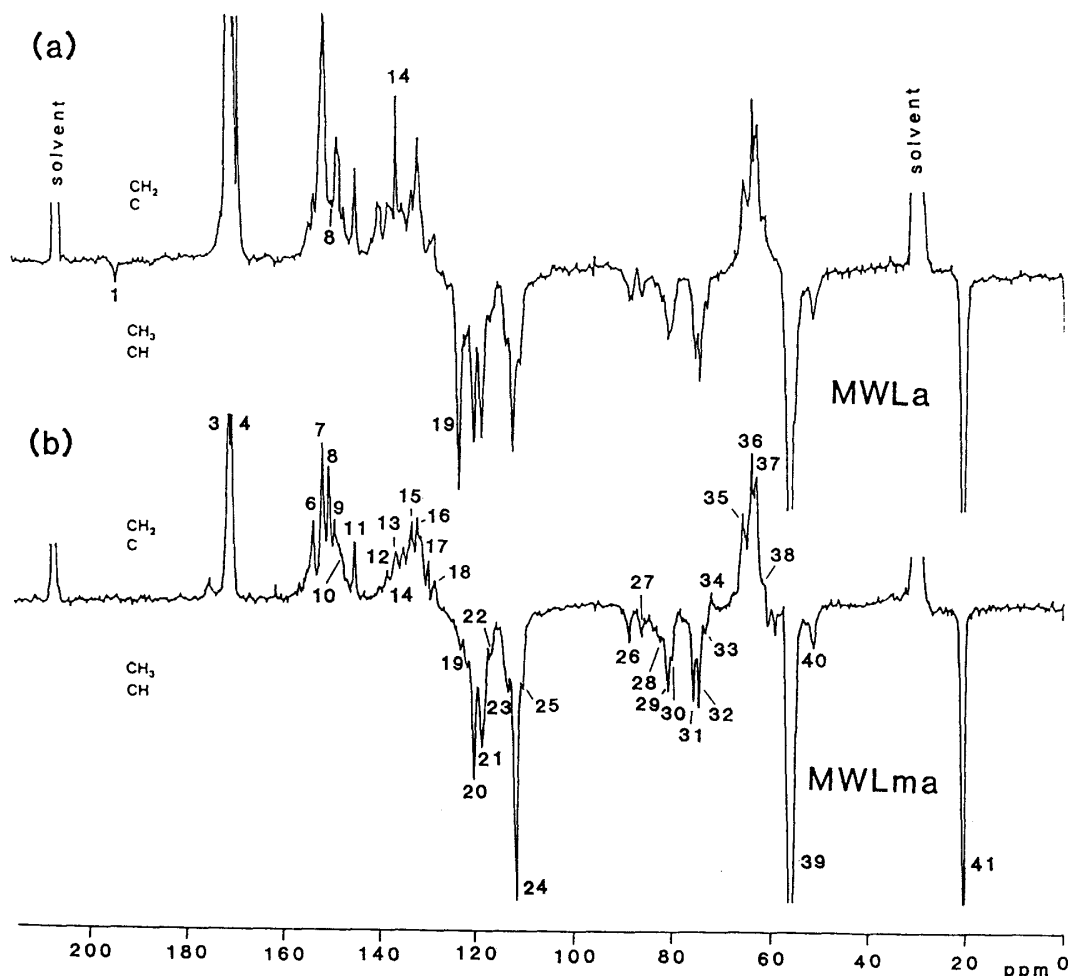


Fig. 3. APT spectra of the acetylated MWL fractions from akamatsu wood. (a): Acetate of MWL fraction (MWLa) (b): Acetate of *p*-methylated MWL fraction (MWLma).

clei, the MWL from akamatsu wood was methylated with diazomethane prior to the acetylation process. The APT spectrum of the resulting MWL acetate (MWLma) is given in Fig. 3, together with the non-methylated fraction (MWLa). The completion of the first methylation step for preparing MWLma was confirmed by disappearance of the aromatic acetoxy-carbonyl signal in the spectrum of MWLma (Fig. 4). From Figure 3, it was clarified that the SP3 side chain signals in the spectrum of MWLma have much the same intensities as those from MWLa, while the intensities in several aromatic carbon signals from MWLma were markedly different from those in the spectrum of MWLa. For instance, the intensities in the signal No. 14 and 19 were remarkably decreased by the pre-treatment, indicating the origins of 3-methoxy-4-acetoxy nuclei. This result is in accordance with the previous assignments^{15,16)}. On the other hand, the intensities of signal No. 6, 8 and 24 were found to be increased because of the formation of 3,4-dimethoxybenzene substructures.

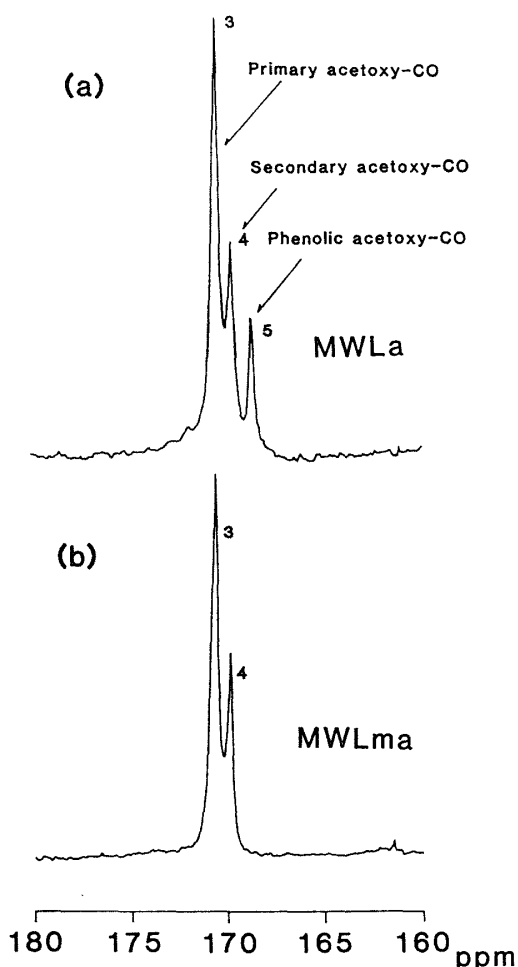


Fig. 4. Acetoxycarbonyl signals of acetylated MWL fractions.
(a): Acetate of MWL fraction (MWLa), (b): Acetate of *p*-methylated MWL fraction (MWLma).

However, it must be noted that the overall assignments for the aromatic carbon atoms in the lignin acetate could not be deduced from this experiment because chemical shifts of ring carbon atoms in 3-methoxy-*p*-etherified lignin model compounds were severely influenced by the steric and inductive effects of their *p*-substituents. For instance, resonance of C-5 in 3-methoxy-4-ethoxybenzene¹⁹⁾ and that of 3-methoxy-4-benzyl-oxybenzene¹⁸⁾ had 1.4 and 2.9 ppm downfield shifts from the resonance of C-5 in 3,4-dimethoxybenzene, respectively. As can be seen from these results, assignments of aromatic carbon atoms in lignins should be achieved by considering the ununiformity of *p*-substituents in the irregular macromolecules.

Multipulse sequences using a spin-echo phenomenon, are effective for discriminating α -ketone from cinnamaldehyde in lignins because the resonance lines from both carbonyl groups have different carbon multiplicities at the same chemical shift region. In fact, we could evidence the existence of the cinnamaldehyde by the downward signal appeared at 194.2 ppm. In addition, reaction of diazomethane with the aldehyde could be confirmed by the disappearance of the downward signal. Because diazomethane is known to react with aldehydes to yield their corresponding methylketones or epoxides²⁰⁾, it is expected that the same type of reaction occurred in the lignin.

4. Conclusions

A convenient J-modulation pulse sequence, Attached Proton Test (APT) technique has been applied to the acetylated milled wood lignin from akamatsu wood. From the APT spectra obtained, it has been clarified that the structural features of lignin acetates could be revealed by the APT method, accompanied by the sufficient signal to noise (S/N) ratio for the routine NMR analyses of lignins.

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